A New Concept on the Mode of Interaction of Narcotic Analgesics with Receptors

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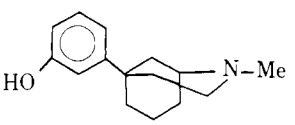
Existing data on the relationship between molecular geometry and narcotic analgesic activity has been interpreted in terms of differing modes of analgesic–receptor interactions. A method for detecting similarities or differences in molecular binding modes has been accomplished by comparing the variation of activity in two or more different series of compounds when identical changes of the N-substituent are made. A parallel change in activity is suggestive of similar binding modes while a nonparallel relationship is indicative of dissimilar interactions. This method has been extended quantitatively to demonstrate the existence of a linear free-energy relationship when binding modes are similar. The value of the slopes from such a relationship are approximately unity. This suggests that, when compounds in two different series interact with the receptors in a similar fashion, identical substituents contribute to the binding by a similar mechanism. The concept of three-point interaction and possible pitfalls inherent in its applicability to the interaction of analgesics with receptors is discussed.

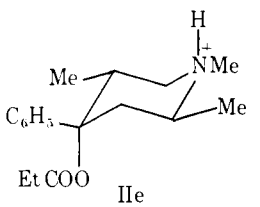
Although it is generally agreed that narcotic analgesics exert their action by interacting with specific receptors, there is little known about the nature of such receptors. One approach to the investigation of receptor topography is the correlation of absolute stereochemistry with analgesic potency. In this connection, the configuration of a variety of optically active structures has been determined.[1-7] A rather perplexing aspect of this problem is that the more active enantiomers of analgesic molecules, having degrees of structural similarity and a common asymmetric center, are not all stereochemically related.

Another phenomenon which has not been adequately rationalized has a bearing on the pharmacophoric conformation(s) of mobile analgesics. It appears that at least two conformations of the phenylpiperidine moiety contained in various compounds are capable of exerting high analgesic potency. For example, the conformationally restricted compound I[8] is as potent as morphine even though the aromatic ring is fixed in the equatorial position. This is in contrast to the phenyl-

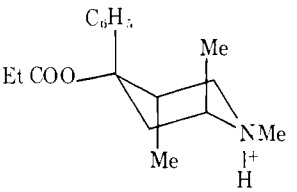


I

piperidine moiety of morphine, where the aromatic group is constrained in the axial conformation. Since I is active, it is conceivable that other phenylpiperidine compounds exert their action in the equatorial conformation when serious nonbonded interactions preclude a significant amount of axial conformer. For example, the change in free energy on going from the equatorial (IIe) to axial conformer (IIa) in the potent analgesic, promedol,[9] is in excess of 7 kcal./mole. Since it seems unlikely that such a large energy barrier can be surmounted as a consequence of an analgesic–receptor interaction, IIe possibly could be a pharmacophoric conformation.



IIe



IIa

In view of the absence of a coherent interpretation of steric factors and other phenomena as related to the mode of interaction of molecules with analgesic receptors, existing structure–activity relationships have been reinterpreted in the light of newer developments in the areas of biological and medicinal chemistry.

The Analgesic–Receptor Interaction.—There are three possible modes of interaction of narcotic analgesics with receptors which merit consideration. Different analgesic molecules may exert their action by: (1) interacting with a single species of receptors, (2) interacting with two or more common species of receptors, and/or (3) interacting with different species of receptors. Since a variety of structures possessing narcotic analgesic activity has been shown to be antagonized by nalorphine,[8,10] it is conceivable that the mode of interaction in certain cases involves either of the first two possibilities. Quantitative studies[11] with analgesic antagonists may provide greater insight into this problem. Recent experiments along these lines have indicated that nalorphine does not necessarily antagonize different analgesics to the same degree.[12] Although quantitative experiments are presently one of the best means of investigating possible modes of interaction of narcotic analgesics with receptors, such studies are open to question since it is not known with certainty whether nalorphine is purely a competitive antagonist. Moreover, since the effects of certain highly potent analgesics[13] do not appear to be completely antagonized by nalorphine, it seems likely that there may be several different species of narcotic analgesic receptors. It ap-

(1) A. H. Beckett in "Progress in Drug Research," Vol. 1, E. Jucker, Ed., Birkhauser Verlag, Basel, 1959, p. 455.
(2) A. H. Beckett, G. Kirk, and R. Thomas, *J. Chem. Soc.*, 1386 (1962).
(3) M. Nakazaki, I. Mita, and N. Toshioka, *Bull. Chem. Soc. Japan*, **36**, 161 (1963).
(4) H. R. Sullivan, J. R. Beck, and A. Pohland, *J. Org. Chem.*, **28**, 2381 (1963).
(5) A. Rheiner, Jr., and A. Brossi, *Experientia*, **20**, 488 (1964).
(6) P. S. Portoghese and D. L. Larson, *J. Pharm. Sci.*, **53**, 302 (1964).
(7) P. S. Portoghese, *J. Med. Chem.*, **8**, 147 (1965).
(8) N. B. Eddy, *Chem. Ind.* (London), 1462 (1959).
(9) I. N. Nazarov, N. S. Prostakov, and N. I. Shvetsov, *J. Gen. Chem. USSR*, **24**, 2798 (1956).
(10) L. A. Woods, *Pharmacol. Rev.*, **8**, 175 (1956).
(11) L. Grumbach and H. I. Chernov, *Federation Proc.*, **20**, 165 (1961).
(12) (a) B. M. Cox, *Brit. J. Pharmacol.*, **22**, 289 (1964); (b) R. M. Veatch, T. K. Adler, and E. L. Way, *J. Pharmacol. Exptl. Therap.*, **145**, 11 (1964).
(13) K. W. Bentley, A. L. A. Boura, A. E. Fitzgraeld, D. G. Hardy, A. McCoubrey, M. L. Aikman, and R. E. Lister, *Nature*, **206**, 102 (1965).

pears, therefore, that all three possibilities are probable and should be considered.

Studies on the interaction of proteins with various types of molecules suggests that there are basic similarities in enzyme specificity, antibody specificity, receptor specificity, and related phenomena.

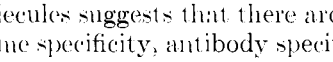
It is now widely believed that the same type of interactions favoring association of hydrocarbon moieties in proteins are also responsible for the binding of small molecules to macromolecules.[14,15] Moreover, it is becoming increasingly evident that the geometry of an active site on an enzyme is not a rigid entity but, rather, possesses a degree of conformational mobility which enables the substrate to be correctly aligned with the catalytic groups necessary for enzyme action.[15] Based on this well-known ability of small molecules to induce conformational changes in proteins, a theory of drug action has recently been proposed.[16]

If one accepts the reasonable assumption that the physicochemical factors responsible for the interaction of an analgesic molecule with a receptor are basically the same as those which cause the binding of small molecules to macromolecules, certain conclusions may be reached regarding the nature of the interaction of analgesic molecules with receptors.

It is proposed that a molecule binds to an analgesic receptor by forming hydrophobic bonds with nonpolar sites and through ion-pair formation[17] with an anionic site. A moiety such as carboxylate or phosphate anion could conceivably be involved in ionic bonding with the protonated amine nitrogen[18] in the analgesic molecule. The binding of the aromatic ring in analgesic molecules has been attributed[1] to van der Waals forces. However, since such attractive forces are highly distance-specific and thought to be effective only in a lock-and-key type of fit,[19] it may be more reasonable to assume that hydrophobic-attractive forces are operative. It is possible that such an attraction involves interaction with an aromatic ring located on the receptor. Such a situation would be analogous to the active site of α-chymotrypsin which contains an aromatic ring[20] that could possibly be involved in the binding of aromatic competitive inhibitors devoid of polar groups.[21] Alkyl moieties in analgesic molecules most probably have the potential of contributing to binding *via* hydrophobic bonds. Since analgesic receptors are capable of exhibiting a high degree of stereoselectivity toward optically active molecules (see Table I), it is conceivable that one enantiomer is more active than its mirror image because of its greater ability to form hydrophobic bonds with sites on the receptor. Although polar groups capable of forming hydrogen bonds with dipolar sites on receptors are not necessary for activity,[22] they could enhance the analgesic effect by increasing the affinity of the molecule. It appears that such dipolar interactions play an important role in controlling the mode[6] of binding of analgesic molecules. This will be covered in detail later in this discussion.

TABLE I

ANALGESIC ACTIVITY OF SOME ENANTIOMERS OF KNOWN CONFIGURATION



No.	R	Enantiomer	ED_{50}[a]	Testing method	Configuration[b]
1	COEt	−	0.8[c]	*d*	(*R*)[c]
		+	25.7		
2	SO_2Et	−	0.8[e,f]	*g*	(*R*)[c]
		+	14		
3	CO_2Et	−	24[h]	*d*	
		+	5.4		(*S*)[c]
4	CHOHEt	(−)-α	3.5[i]	*d*	(6*S*)
		(+)-α	24.7		
5	CHOHEt	(−)-β	7.6[i]	*d*	(6*R*)
		(+)-β	63.7		
6	CHOAcEt	(−)-α	1.8[i]	*d*	
		(+)-α	0.3		(6*R*)
7	CHOAcEt	(−)-β	0.4[i]	*d*	(6*R*)
		(+)-β	4.1		
8	$(C_4H_3S)_2C{=}CHCHNMe_2$ (Me)	−	12[f,j]	*g*	
		+	1.2		(*R*)[c]
9	PhNCH$_2$CHNCH$_2$Ph (COEt, Me, Me)	−[k]	Inactive at 50[l,m]	*g*	
		+	4.3		(*S*)[n]
10	PhNCH$_2$CHNCH$_2$CH$_2$Ph (COEt, Me, Me)	−[k]	11.7[l,m]	*g*	
		+	3.6		(*S*)[c]

[a] Mg./kg. administered subcutaneously. [b] Configuration of more active enantiomer. [c] D. G. Leimbach and N. B. Eddy, *J. Pharmacol. Exptl. Therap.*, **110**, 135 (1954). [d] Contact heat on feet of mice. [e] A. H. Beckett and A. F. Casy, *J. Chem. Soc.*, 900 (1955). [f] Converted to ED_{50}. [g] Heat on rat tail. [h] Ref. 38. [i] N. B. Eddy, E. L. May, and E. Mosettig, *J. Org. Chem.*, **17**, 321 (1952). [j] A. F. Green, *Brit. J. Pharmacol.*, **8**, 2 (1953). [k] Optical rotation of free base. [l] W. B. Wright, Jr., and R. A. Hardy, Jr., *J. Med. Chem.*, **6**, 128 (1963). [m] AD_{50}. [n] Ref. 6.

Analgesic receptors having a degree of flexibility[15] would allow interaction with a greater variety of analgesic molecules than would rigid receptors. This could, in part, account for the fact that narcotic analgesic activity can be found in compounds that do not have similar geometry. For example, it is known that conformationally restricted phenolic compounds (Table V) possessing equatorial and axial aromatic rings exhibit comparable activity even though the distance between ring and basic nitrogen is different. Inasmuch as conformational restriction precludes the possibility that the molecules are assuming a similar conformation, it is conceivable that induced fit is a factor in this case.

The question of whether narcotic analgesics are interacting directly with sites on the nervous tissue or by an indirect mechanism is not yet known. In any case, however, analgesic molecules most probably are involved in binding to some type of macromolecular structure located in the central nervous system.[23] Association of analgesic molecules with receptors

(14) W. Kauzmann, *Advan. Protein Chem.*, **14**, 1 (1959).

(15) D. E. Koshland, Jr., Proceedings of the First International Pharmacological Meeting, Stockholm, 1961; Vol. 7, Pergamon Press Ltd., London, 1963, p. 161, and references cited therein.

(16) B. Belleau, *J. Med. Chem.*, **7**, 776 (1964).

(17) A. H. Beckett, *J. Pharm. Pharmacol.*, **8**, 848 (1956).

(18) S. H. Biers and G. W. Stevenson, *Federation Proc.*, **19**, 272 (1960).

(19) B. Belleau and G. Lacasse, *J. Med. Chem.*, **7**, 786 (1964).

(20) M. L. Bender, J. V. Killheffer, Jr., F. J. Kezdy, *J. Am. Chem. Soc.*, **86**, 5331 (1964).

(21) J. L. Miles, D. A. Robinson, and W. J. Canady, *J. Biol. Chem.*, **238**, 2932 (1963).

(22) O. J. Braenden, N. B. Eddy, and H. Halbach, *Bull. World Health Organ.*, **13**, 937 (1955).

(23) L. T. Angeles, F. W. Schueler, P. R. T. Liu, and A. Sotto, *Arch. intern. pharmacodyn.*, **152**, 253 (1964).

located on these macromolecules may promote a rearrangement of the receptor substance which contributes to the binding process.[15] The resulting macromolecular perturbation[16] ultimately may lead to an analgesic effect. It is quite possible that several different types of complexes could give rise to the pharmacological effect. If the macromolecular rearrangement does not lead to analgesia, then the compound may act as a competitive antagonist (*i.e.*, in rats and mice). Inactivity can also be caused by a lack of affinity[24] for the receptor due to unfavorable hydrophobic interaction. It is likely that the value of the ED_{50} is dependent both on the ability of a molecule to induce a proper macromolecular perturbation[16] and on the affinity for the receptor.

Configurational Selectivity of Analgesic Receptors.—The stereoselectivity of analgesic receptors toward a variety of narcotic analgesics is amply documented (Table I). In all cases activity is distributed unequally in enantiomeric molecules. It is significant that the more active enantiomers are not all configurationally related. For example, methadone (**1**) and thiambutene (**8**) are related to (*R*)-alanine while the carbethoxy analog of methadone (**3**), diampromide (**10**), and α-methadol (**4**) are of opposite configuration. The aforementioned cases are quite obvious because of the inversion in configurational selectivity of the analgesic receptors. Quite often, however, the selectivity of the receptors, though not inverted, is changed considerably when groups in a molecule are altered (see Table I).

In compounds structurally related to methadone, alteration of the ketonic carbonyl group causes profound changes in the enantiomeric potency ratio. For instance, reduction of methadone to α-methadol (**4**) followed by transformation to the acetate derivative (**6**) causes the (*R*), (*S*), and (*R*) isomers of **1**, **4**, and **6**, respectively, to be more active. A mere change in relative stereochemistry can also influence the antipodal discriminatory power of the receptors. Thus, the more active enantiomer of α-methadol (**4**) is in the (*S*) series while the more potent optical antipode of β-methadol (**5**) possesses the (6*R*) configuration.

Variations in the discriminatory aptitude of analgesic receptors toward various enantiomers can be caused by several factors which may be important singly or in combination. These are as follows: (1) Differences in the course of binding of stereochemically related compounds to a single species of receptors. Figure **1** illustrates schematically how two different molecules of opposite configuration may interact with an analgesic receptor. Such differences in the binding mode will be manifested by an inversion of the configurational selectivity of the receptor. Dipoles conceivably can be sites which are hydrogen bonding proton donors (X) or acceptors (Y). As illustrated, interaction of methadone with an analgesic receptor may involve hydrogen bonding of the ketonic carbonyl group by X, whereas with α-methanol, OH···Y may occur. Hence, alteration of a polar group on an analgesic molecule can afford a compound which may interact with dipolar sites and hydrophobic areas that differ from those involved in the binding of the unaltered structure.[6] (2) Differences in the partitioning of configurationally related molecules on two or more common species of receptors. Hence, if two analgesics are partitioned in different proportions on two or more common receptors which do not have the same stereoselectivity, an apparent change in the stereoselectivity of the receptors will result. (3) Binding of stereochemically related compounds to different species of receptors having dissimilar configurational selectivity.

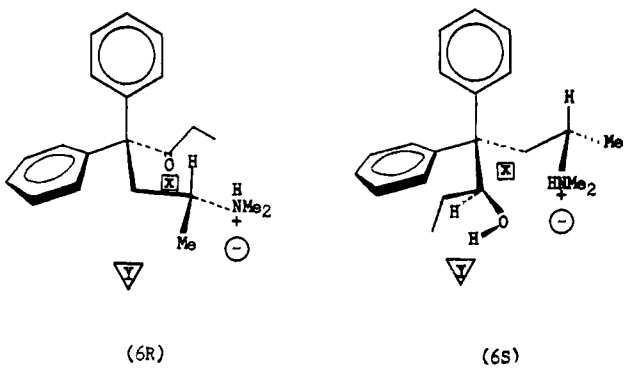


Figure 1.—An illustration of how different polar groups in analgesic molecules may cause inversion in the configurational selectivity of an analgesic receptor. Hydrogen bonding proton donor and acceptor dipoles are denoted by x and y, respectively. The anionic site is represented by −.

It may be possible to make a distinction between the first two cases and the third possibility by studies with analgesic antagonists. However, it may prove difficult to distinguish between (1) and (2) by such a method. The situation may be more complex if there are combinations of case (2) and (3).

Inasmuch as no quantitative studies with analgesic antagonists have been carried out on compounds such as α-methadol and the basic anilide analgesics [whose more active enantiometers are all stereochemically related to (*S*)-alanine], firm conclusions regarding the mode of interaction of these compounds with analgesic receptors cannot presently be made.

It is of interest that inversion in the configurational selectivity of muscarinic receptors toward muscarone[25] has been suggested to be caused by binding of muscarine and muscarone with different portions of the receptor.[26] Similar behavior has been displayed by α-chymotrypsin in the hydrolysis of various asymmetric esters.[27,28] These observations have likewise been interpreted as being due to differences in the interaction of substrates with the active site.[28-30]

Variation of N-Substituents as a Means of Comparing Modes of Drug–Receptor Interaction.—It is known that variation in the substituent attached to the basic nitrogen of an analgesiophore[31] can exert a profound influence on activity.[32] For example, replacing a methyl with a cinnamyl group in 4-phenyl-4-propionoxypiperidine results in a hundredfold increase in analgesic activ-

(24) E. J. Ariens, "Molecular Pharmacology," Vol. 1, Academic Press Inc., New York, N. Y., 1964, p. 232.

(25) P. G. Waser, *Pharmacol. Rev.*, **13**, 465 (1961).

(26) B. Belleau and J. Puranen, *J. Med. Chem.*, **6**, 325 (1963).

(27) G. E. Hein, R. B. McGriff, and C. Niemann, *J. Am. Chem. Soc.*, **82**, 1830 (1960).

(28) S. G. Cohen and S. Y. Weinstein, *ibid.*, **86**, 5326 (1964).

(29) I. B. Wilson and B. F. Erlanger, *ibid.*, **82**, 6422 (1960).

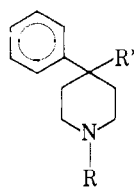
(30) E. S. Awad, H. Neurath, and B. S. Hartley, *J. Biol. Chem.*, **235**, PC35 (1960).

(31) Analgesiophore is defined as the analgesic molecule less the substituent on the basic nitrogen.

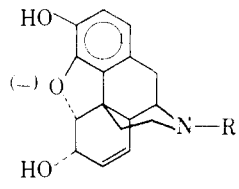
(32) P. A. Janssen and N. B. Eddy, *J. Med. Pharm. Chem.*, **2**, 31 (1960).

TABLE II

RELATIVE ANALGESIC ACTIVITY OF STRUCTURES CONTAINING THE PHENYLPIPERIDINE MOIETY



1, R' = CO_2Et
2, R' = OCOEt
3, R' = OCOMe



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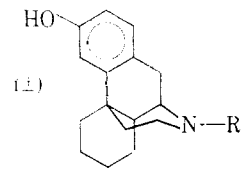

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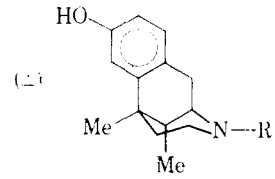

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R	Relative activity[a] 1[32,b,c] H.P.[i]	1 R.H.[j]	2[32,d] H.P.[i]	2 R.H.[j]	3[32,d] H.P.[i]	3 R.H.[j]	Relativity activity[e] 4[33] R.H.[j]	5[f,g] H.P.[i]	6[g,h] H.P.[i]
Me	1.0	1.0	7.4	26	1.0	2.4	1.0	2	0.7
Et		0.5[k]					<0.1	0.1	0
n-Pr		1.5[k]					11		0
Allyl		0.8[k]					<0.1	11	
n-Bu		1.5[k]					<0.1		11
n-Amyl		1.5[k]					0.7	~2	~1
$C_6H_5CH_2$	<0.3	<0.5	1.5	1.4	1.0	1.1	<11.1	11	
	0.15	0.32	3.8						
$C_6H_5(CH_2)_2$	2.3	2.6	25	110	12	60	15	~10	~11
	2.7		66	69	66	72			
$C_6H_5(CH_2)_3$	23	20	162	572	62	265			11.15
	27	18	318	637	90	142			
$C_6H_5(CH_2)_4$	1.6	2.8	54	108	32	39		~0.3	
$C_6H_5CH{=}CHCH_2$	32	40	261	1100	82	376	<0.1	0	
	61	39	650	785		189			

[a] Analgesic activity relative to meperidine: a value of 10 signifies the compound is 10 times more potent than the reference compound. Unless otherwise specified compounds were administered subcutaneously. [b] R. H. Thorp and E. Walton, *J. Chem. Soc.*, 559 (1948). [c] B. Elpern, L. N. Gardner, and L. Grumbach, *J. Am. Chem. Soc.*, **79**, 1951 (1957). [d] B. Elpern, W. Wetteran, P. Carbateas, and L. Grumbach, *ibid.*, **80**, 4916 (1958). [e] Analgesic activity relative to morphine in the same sense as in *a*. [f] N. B. Eddy, H. Besendorf, and B. Pellmont, *Bull. Narcotics, U. N. Dept. Social Affairs*, **10**, 23 (1958). [g] L. B. Mellett and L. A. Woods "Progress in Drug Research," Vol. 5, E. Jucker, Ed., Birkhauser Verlag, Basel, 1963, p. 157. [h] J. H. Ager and E. L. May, *J. Org. Chem.*, **25**, 984 (1960). [i] Hot plate method using mice. [j] Rat tail radiant heat method. [k] Administered intraperitoneally.

ity. An identical substituent change in the morphine series results in loss of activity.[33] The fact that N-cinnamylnormorphine is not an antagonist[33] suggests that its inactivity may, in part, be due mainly to lack of affinity for the receptors. A plausible explanation for this nonparallel relationship is that the cinnamyl substituents attached to the aforementioned analgesiophores are in different physiochemical environments on the receptors. This would mean that the binding mode of the analgesiophore in phenylpiperidine compounds is different from that in the morphine series.

Of greater significance is the report that N-allylnormeperidine (Table II, **1**, R = allyl), unlike nalorphine (**4**, R = allyl) which behaves as a morphine antagonist and is virtually inactive (*i.e.*, in mice or rats), is not an antagonist[34] and possesses activity comparable to meperidine. This suggests that the N-allyl derivatives of **1** and **4** (Table II) are complexing with the analgesic receptors by dissimilar modes. Since the affinity of nalorphine for the receptors has not been diminished, the great variance in the pharmacological effect may be reflective of a difference in binding mode. Such differences in the mode of interaction can be attributed to any of the factors which have been discussed in connection with the inversion of configurational selectivity of analgesic receptors.

Although an analgesiophore in one series of compounds may interact with analgesic receptors in a manner which is dissimilar to a different analgesiophore containing an identical N-substituent, it is reasonable to assume that, regardless of the binding mode, all analgesics (including competitive antagonists) are involved in ionic bonding with an identical anionic site. The anionic site may be envisaged as a pivotal point around which various modes of binding may occur (Figure 2). This does not necessarily imply that 360° surrounding the anionic site is available for binding; it may be only a small fraction of this area. The structural features of an analgesiophore determine the position of binding of an N-substituent to a single species, to two or more common species, and/or to different species of receptors. It is also conceivable that the N-substituent may be capable of modifying the binding mode of the analgesiophore. If this is the case, each analgesic in a series containing the same analgesiophore and different N-substituents would exert its action *via* slightly different binding modes.

When different analgesiophores interact with receptors by similar modes, then identical N-substituents should contribute to the pharmacological effect by the same mechanism. If each compound in one series of analgesics is compared with a member in a second series which has a different analgesiophore and an identical N-substituent, then it may be possible to determine whether the binding modes of the analgesiophores (attached to the same N-substituent) are similar or quite

(33) C. A. Winter, P. D. Oralkovats, and E. G. Lehman, *Arch. intern. pharmacodyn.*, **110**, 186 (1957).

(34) P. J. Costa and D. D. Bonnycastle, *J. Pharmacol. Expll. Therap.*, **113**, 310 (1955).

different. Thus, if identical changes in the N-substituent in two series of compounds produces parallel changes in potency, the mode of binding of the analgesiophores should be similar. A relationship which has a high degree of nonparallel activity is suggestive of binding modes which are quite dissimilar.

The activity of identically N-substituted analgesiophores has been compiled in Table II. It can be seen that the meperidine series (**1**) and identically substituted reversed esters (series **2** and **3**) exhibit parallel changes in activity. Comparison of the aforementioned series with identically substituted compounds related to morphine (**4**, **5**, and **6**) show no parallel relationship. On the other hand, derivatives of morphine (**4**), morphinan (**5**), and benzomorphan (**6**) do display parallel changes in potency.

The correlations in Table II suggest that the analgesiophores (containing identical N-substituents) in series **1**, **2**, and **3** are binding to the receptor by similar modes and that an analogous situation exists among the structures related to morphine (series **4**, **5**, and **6**). Furthermore, the high degree of nonparallel activity between phenylpiperidine derivatives (series **1**, **2**, and **3**) and morphine-like compounds (series **4**, **5**, and **6**) strongly suggests that the binding modes of identically substiuted compounds in the former series may be quite different from the latter.

If identically substituted compounds in two different series are interacting with receptors in a similar manner, then the quantitative contribution of various substituents to the analgesic effect should produce, under steady state conditions, proportionate variations of activity in both series. If a point is plotted whose abscissa is the logarithm of the activity for the appropriately substituted compound in one series and whose ordinate is the logarithm of the activity in an identically substituted compound in the second series, the resultant points should describe a straight line. Such a proportionality is known as a linear free-energy relationship.[35] Linearity would be a consequence of similar binding modes of two different analgesics containing identical N-substituents and would be due to the fact that the change in $\Delta S = 0$ or that variations in ΔS are proportional to changes in ΔH. If the modes of interaction are quite dissimilar, then identical N-substituents attached to two different analgesiophores would experience different physicochemical environments on the receptors. Such a situation would give rise to nonproportionate differences in ΔS and ΔH which would be manifested by a nonparallel relationship and a scatter of points. If the binding modes of two different analgesiophores containing identical substituents are similar, the slope of the regression should be in the vicinity of **1**. This, of course, is dependent on the assumption that, prior to the drug–receptor interaction, identical substituents on two different analgesiophores will affect the biodistribution of the compounds in a similar fashion. This assumption is quite reasonable in view of the successful application of substituent constants[36] for the purpose of predicting drug availability at the site of action. Thus, parallelism or nonparallelism of potency is interpreted as a manifestation of events at the receptor level. When the

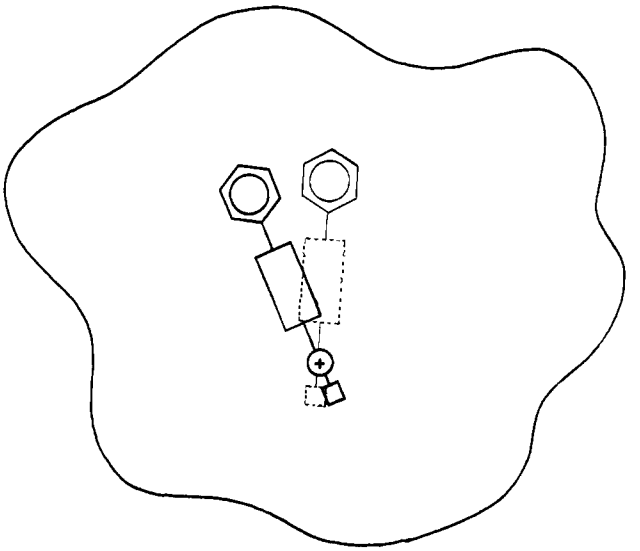


Figure 2.—A schematic illustration of two different positions of binding to a receptor. The protonated amine nitrogen is represented by + and the square denotes an N-substituent. The anionic site lies directly beneath +.

mode of binding is not similar, scattering of points may make the value of the slope indeterminate. If several dissimilar binding modes exist for each of two different analgesiophores having the same N-substituent, depending on the relative population of the modes, degrees of point scattering may be observed. As differences in the modes of interaction increase, the standard deviation of points which comprise the regression will become quite large and result ultimately in a nonparallel relationship.

Janssen and Eddy[37] have reported on the analgesic activity of several series (Table II, series **1**, **2**, and **3**) of identically N-substituted phenylpiperidine derivatives. Since their data are reported with well defined confidence limits, it seemed that they could be used to demonstrate the existence of a linear free-energy relationship. An additional advantage was that regressions obtained from the data of Janssen could be compared with those of Eddy.

Although the least-square regressions obtained from both Janssen's and Eddy's data (Table III) represent a first approximation due to the limited number of compounds tested, it can be seen that the values of the slopes and the linear correlation coefficients are fairly close to unity. This is consistent, in a way which is more exacting than qualitative examination, with the idea that different analgesiophores containing the same substituent are interacting with the analgesic receptors in a similar manner. It is evident that the standard error (S.E.) and the standard deviation (S.D.) for each of two regressions (which possess identical analgesiophores) obtained from two different sources are in good agreement. A plot of the logarithm of the activity (μM/kg.) for series **1** and series **2** is shown in Figure 3.

Since S.D. represents a measure of point scatter it should give an indication of the degree of similarity in the binding of different analgesiophores to the receptors. If the data in Table III are significant, the substantially higher values of S.D. in regressions de-

(35) H. H. Jaffe, *Chem. Rev.*, **53**, 191 (1953).
(36) C. Hansch and T. Fujita, *J. Am. Chem. Soc.*, **86**, 1616 (1964).
(37) P. A. J. Janssen, "Synthetic Analgesics," Part I, Pergamon Press Inc., New York, N. Y., 1960, p. 63.

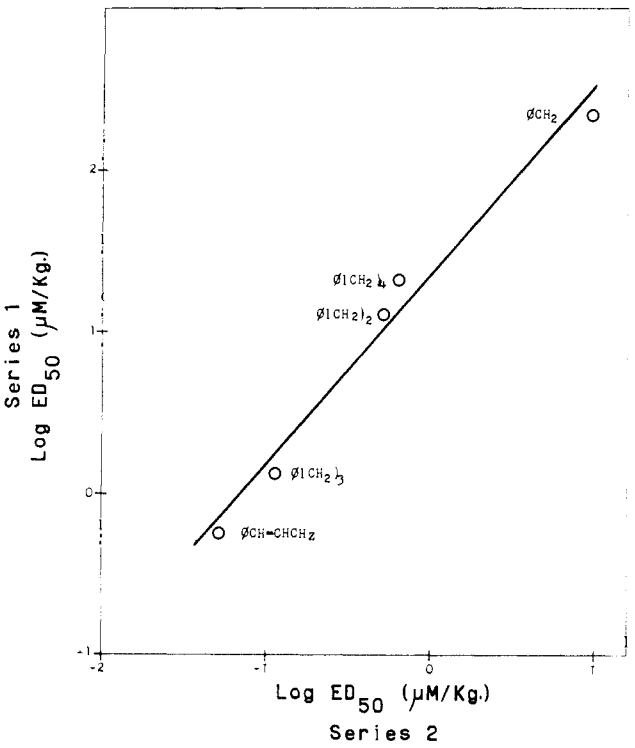


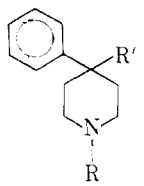
Figure 3.—A plot of the log ED_{50} of analgesics in series **1** *vs.* the log ED_{50} of identically substituted compounds in series **2**.

rived from **1** *vs.* **3** may mean that the analgesiophore in series **3** binds to the receptors in a manner which has less of an element of similarity than do **1** and **2**.

Although series **4**, **5**, and **6** (Table II) show parallel changes in potency, the pharmacological data on these structures have been obtained from several sources. Consequently, a quantitative correlation of these series is not presently feasible. The data on open-chain analgesics (Table IV) present the same problem and have the added disadvantage that changes in the N-substituent are not of the type which cause large variation in activity. Therefore, only substantial changes in analgesic activity may be meaningfully interpreted when identically substituted compounds in various series are compared. Furthermore, since series **3** and **4** do not have many substituents in common with other series in Table IV, any conclusions drawn from these data regarding the aforementioned series can be considered only tentative. In spite of these limitations, a certain amount of information can be extracted from these data. Qualitative inspection of these series indicates that **1**, **2**, **3**, and **5** may have a degree of parallel activity. This could possibly mean that compounds in the above series are interacting with analgesic receptors by similar modes. The fact that the more active enantiomers in series **2**, **3**, and **5** (R = R′ = Me) are configurationally related is consistent with the above conclusion. It can be seen that the compounds present in series **4** and **6** show little correlation with series **2**, **3**, and **5**. Significantly, the more active enantiomers in series **4** (R = R′ = Me) and **6** (R = Me; R′ = benzyl) possess the opposite configuration. In this case, dissimilar binding modes are reflected both by inversion in the configurational selectivity of the analgesic receptors and by nonparallel changes in activity. It is important to point out that an identical stereochemical

TABLE III

REGRESSION ANALYSIS OF THE LOGARITHM OF THE ANALGESIC ACTIVITY IN VARIOUS N-SUBSTITUTED PHENYLPIPERIDINE SERIES



1, R′ = CO_2Et
2, R′ = OCOEt
3, R′ = OCOMe

Correlation[a]	Slope[b]	S.E.	S.D.	r[c]	n[d]	Data source[e]
1 *vs.* **2**[f]	1.20	0.09	0.16	0.99	5	*g*
	1.01	0.13	0.25	0.96	6	*h*
1 *vs.* **3**[i]	0.93	0.24	0.37	0.90	4	*g*
	0.87	0.21	0.42	0.87	6	*h*
3 *vs.* **2**[j]	1.09	0.20	0.27	0.96	4	*g*
	1.06	0.19	0.25	0.94	5	*h*

[a] Series **1**, **2**, and **3** were plotted as the logarithm of the activity (μM/kg.). [b] Values were calculated by the method of least squares. [c] Represents the linear correlation coefficient; when $r = 1$ there is a perfect correlation; if $r = 0$ there is no correlation. [d] Denotes the number of points in the regression. [e] All data were obtained from ref. 32; mice were the test animals. [f] R = $C_6H_5CH{=}CHCH_2$, $C_6H_5(CH_2)_3$, $C_6H_5(CH_2)_2$, $C_6H_5(CH_2)_4$, $C_6H_5CH_2$; R = $C_6H_5CH(OCOEt)CH_2CH_2$, $C_6H_5CH{=}CHCH_2$, $C_6H_5(CH_2)_3$, $C_6H_5CH{=}CH(CH_2)_2$, CH_3. [g] Eddy's data. [h] Janssen's data. [i] R = $C_6H_5(CH_2)_3$, $C_6H_5(CH_2)_2$, $C_6H_5(CH_2)_4$, $C_6H_5CH_2$; R = $C_6H_5CH(OAc)CH_2CH_2$, $C_6H_5CH{=}CHCH_2$, $C_6H_5(CH_2)_3$, $C_6H_5(CH_2)_2$, $C_6H_5CH{=}CH(CH_2)_2$, CH_3. [j] R = $C_6H_5(CH_2)_3$, $C_6H_5(CH_2)_2$, $C_6H_5(CH_2)_4$, $C_6H_5CH_2$; R = $C_6H_5CH{=}CHCH_2$, $C_6H_5(CH_2)_3$, $C_6H_5(CH_2)_2$, $C_6H_5CH{=}CH(CH_2)_2$, CH_3.

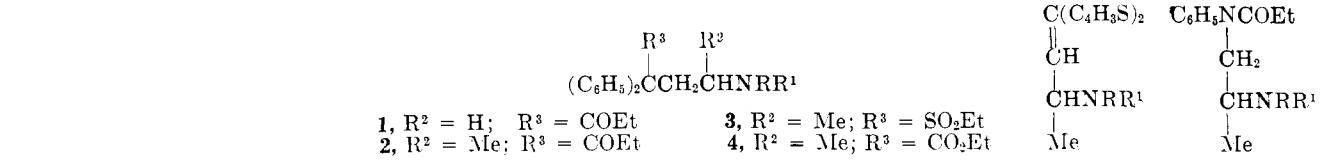
relationship alone does not necessarily indicate that analgesic molecules are interacting with receptors in a similar fashion since such a relationship may be coincidental rather than reflecting similar binding. A more rigorous procedure would involve the use of substituent variation and stereochemical data. For example, the more active enantiomers of the carbethoxy analog of methadone (Table I, **3**) and the basic anilide compounds (Table I, **9** and **10**) possess the same configuration but do not appear to be similarly affected by identical N-substituents. This suggests different binding modes despite the fact that the more active optical isomers are stereochemically related.

Influence of a Phenolic Hydroxyl Group on Molecular Modes of Interaction with Analgesic Receptors.—The ability of a *meta* phenolic hydroxyl group to enhance analgesic activity in compounds containing a basic nitrogen located in a ring is well known.[8,22,38] Placement of the hydroxyl group in the *ortho* or *para* position causes a great decrease or loss of activity.[22] If the aromatic ring is nonphenolic, then activity is usually decreased but not lost. It is conceivable that such behavior is caused by the interaction of the *meta* OH with a dipolar site on an analgesic receptor which aids in binding of the molecule. An *ortho* or *para* hydroxyl group may be responsible for incorrectly aligning the molecule which could result in inactivity due to loss of affinity for the receptors. Since nonphenolic analgesics should not be influenced by a dipolar site which has the potential for binding a pheno-

(38) N. B. Eddy, H. Halbach, and O. J. Braenden, *Bull. World Health Organ.*, **14**, 353 (1956).

TABLE IV

RELATIVE ANALGESIC ACTIVITY[a,b] OF STRUCTURES RELATED TO METHADONE



1, R^2 = H; R^3 = COEt
2, R^2 = Me; R^3 = COEt
3, R^2 = Me; R^3 = SO_2Et
4, R^2 = Me; R^3 = CO_2Et

R	R^1	**1**[c]	**2**[c]	**3**[c]	**4**[c]	**5**[d]	**6**[e]
Me	Me	1.2,[f] 2.5[f,g]	7.8,[f] 5.6[f,g]	6	1.3,[f] 0.55[g]	5	0
Me	Benzyl	0, 0[g]				<0.1	1.4[h]
Et	Et	0.8,[f] 0.82[g]	8.1			5	0
n-Pr	*n*-Pr	<0.33, <0.25[g]				<0.1	
Allyl	Allyl	0.5[i]				0.7	
C_4H_8		4.0, 1.9[f,g]	4,[j] 5.7[f,g]			3.5	0
C_4H_8O		7.0, 4.5[f,g]	19,[f] 8.5[f,g]		<0.1[g]	1	0
C_5H_{10}		2.6,[f] 2.5[f,g]	20, 5.4[f,g]	6	0.2[g]	5.5	0

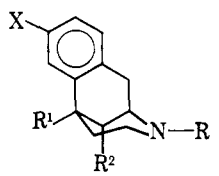
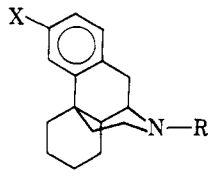
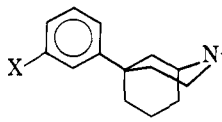
[a] Analgesic activity relative to meperidine; a value of 10 signifies the compound is 10 times more potent than the reference compound. [b] Unless otherwise specified compounds were administered subcutaneously to rats. [c] Data were obtained from ref. 37, Table V, p. 63. [d] Values were calculated from footnote *j*, Table I. [e] With the exception of the methyl benzyl analog, all compounds in this series were inactive at 25 mg./kg. We thank Dr. W. Wright, Jr., for providing this information. [f] Average value. [g] Mice were employed as test animals. [h] Footnote *l*, Table I. [i] Animal species not revealed. [j] Administered intraperitoneally.

lic OH group, the mode of binding of phenolic and nonphenolic compounds may sometimes be quite dissimilar.

Data on phenolic and nonphenolic analgesics (Table V) show that replacing the methyl with a phenethyl substituent causes an enhancement of analgesic activity in phenolic morphinans and benzomorphans, whereas compounds containing no hydroxyl group exhibit a decrease in potency when an identical change in substituent is made. Since phenolic and nonphenolic compounds do not exhibit parallel changes in activity, it is probable that the modes of binding are not the same. Inasmuch as the aforementioned compounds are conformationally immobile, it appears that although molecular shape is an important criterion, it is not the only factor which determines the mode of binding. The same factors which have been discussed in connection with the variation in the enantiomeric potency ratio of various analgesics (Table I) may also be of importance here. Interestingly, the azabicyclo[3.3.1]nonane derivatives (Table V) do show parallel changes in activity.

TABLE V[a]

ANALGESIC ACTIVITY OF PHENOLIC AND NONPHENOLIC COMPOUNDS

Structure	X	R	R^1	R^2	ED_{50}[a]
	H	Me	H	H	18.7
	H	$CH_2CH_2C_6H_5$	H	H	31.9
	OH	Me	Me	H	8.9
	OH	$CH_2CH_2C_6H_5$	Me	H	0.4
	OH	Me	Me	Me	2.6
	OH	$CH_2CH_2C_6H_5$	Me	Me	0.1
	H	Me	—	—	7.3
	H	$CH_2CH_2C_6H_5$	—	—	38.7
	OH	Me	—	—	0.5
	OH	$CH_2CH_2C_6H_5$	—	—	0.2
	H	Me	—	—	18.4
	H	$CH_2CH_2C_6H_5$	—	—	28.7
	OH	Me	—	—	2.0
	OH	$CH_2CH_2C_6H_5$	—	—	3.99

[a] Dose (mg./kg.) administered subcutaneously to mice.

This could be coincidental or it may be indicative of very similar binding modes. Additional data would be needed in order to distinguish between these alternatives.

The Concept of Three-Point Interaction as Related to Analgesic Receptors.—The useful concept of three point interaction[1,24] has been employed in explaining the antipodal selectivity of both enzymes and drug receptors. This type of analysis, however, should be applied with caution as it represents an oversimplified version of a relationship which can become quite complex. Thus, it has been observed that the configurational selectivity of muscarinic receptors[31,32] and of α-chymotrypsin[34–36] is dependent on the characteristics of polar and nonpolar groups which are a part of the drug or substrate. Such phenomena have been explained in terms of dissimilar interaction with the receptor or active site. It is possible that an analogous situation exists for analgesic receptors, in that different sites on the same receptors have the potential for binding some common features of structurally dissimilar analgesiophores. Furthermore, if more than one species of receptors having dissimilar steric requirements are interacting with analgesic molecules, the observed configurational selectivity will represent an average value rather than a single type of analgesic–receptor interaction. Such differences in the mode of binding of optically active narcotic analgesics are usually reflected by variations in the enantiomeric potency ratio. In certain cases (Table I, **3**, **4**, **9**, and **10**) this may involve an apparent inversion in the configurational selectivity of the receptors. This means that stereochemical data cannot be used as the sole criterion in determining whether binding modes are similar, although it may be employed in the detection of dissimilar modes of interaction. As has been pointed out previously in this communication, *an identical absolute stereochemistry of the more active analgesic enantiomers does not necessarily indicate that these structures are interacting with analgesic receptors by similar modes unless variation of the N-substituent produces at least a roughly qualitative parallel change in activity.* Moreover, the data presented in Tables II and IV suggest that it may be misleading to assume, for example, that methadone, meperidine, and morphine all exert their action by interacting with receptors in very similar

pharmacophoric conformations. In comparing the mode of binding of various narcotic analgesics, one must consider not only the geometric disposition of pharmacophoric moieties but also take into account the role played by other groups which are not deemed essential for activity. If quite a different pattern of contributions to drug–receptor binding are made by various configurationally related analgesic molecules, the conclusions drawn from the three-point interaction concept to rationalize configurational selectivity of receptors may not necessarily be valid.

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Stereochemical Factors Related to the Potency of Anticholinergic Psychotomimetic Drugs

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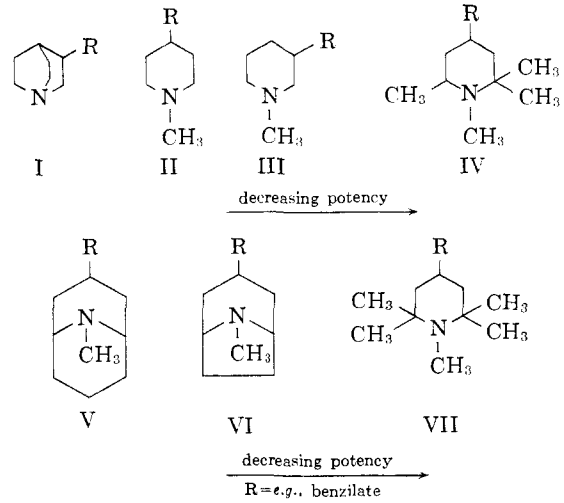
A group of glycolate esters of heterocyclic amino alcohols have been examined from the point of view of stereochemistry in an effort to correlate pharmacological action with molecular configuration. The examination was confined to the properties of the heterocyclic amino group and was conducted with respect to the psychotomimetic properties of the drugs. An important factor for psychotomimetic potency was the availability of the electron pair on nitrogen, which, presumably, must combine with an electrophilic center of the biological receptor site. Conformational factors were important insofar as they contributed to 1,3-diaxial interference by axial hydrogen atoms. Intramolecular interactions involving the nonbonded electron pair on nitrogen were believed to diminish potency.

Considerable interest has been focussed on the structure–activity relationships of substituted glycolate esters of heterocyclic amino alcohols which possess marked psychotogenic (*i.e.*, psychotomimetic) properties.[1,2] Previous studies were devoted largely to the piperidyl and pyrrolidyl esters, with emphasis on variation in the acid moiety of the esters. Since it was known that variations in the type of heterocyclic amino alcohol resulted in striking differences in psychotomimetic effectiveness, an opportunity was at hand to examine the possible role of the amine moiety in the pharmacological action of this series of drugs.



Pharmacology.—The drugs included in this study were evaluated for their anticholinergic potency and for their effect on those parameters of behavior which are believed to be related to the psychotomimetic action of the agents in humans. A measurement of hyperactivity in rats has been found to be a simple and effective means of evaluating the over-all action of the drugs upon the central nervous system.[1,2] A more direct measurement of the drugs' disturbance of higher central nervous function can be made by means of the swim maze and "peek" tests.[3,4] A description of these tests and their reliability in predicting the psychotomimetic action of the glycolate esters in humans has been described in detail elsewhere.[1,2] Briefly the swim maze consists of a maze which contains water in which a mouse is compelled to swim in order to escape drowning; the more centrally active drugs produce a greater number of errors in performance. The "peek" test was developed by Kosman[4] as a means of quantitating peculiar head-bobbing and head-swaying movements associated with the centrally active glycolate esters,[2] and although the significance of this test in determining the behavioral aberrations of the drugs is not too well understood, it has proved to be extremely effective in predicting psychotomimetic potency in humans. The ability of the drugs to block the acetylcholine-induced contraction of the isolated rabbit ileum was taken as a measure of anticholinergic potency.[5]

(1) L. G. Abood and J. H. Biel *Intern. Rev. Neurobiol.*, **4**, 217 (1962).
(2) L. G. Abood, A. Ostfeld, and J. H. Biel, *Arch. intern. pharmacodyn.* **120**, 186 (1959).
(3) M. E. Kosman, *Proc. Soc. Exptl. Biol. Med.*, **115**, 728 (1964).
(4) M. E. Kosman, *ibid.*, in press.
(5) H. C. Chang and J. H. Gaddum, *J. Physiol.*, **79**, 255 (1933).